Macromolecular modulation in a tissue-to-tissue model system differentially regulates the behaviour of hBMSCs

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INTRODUCTION: The simultaneous creation of multiple tissues and their functional assembly leading to complex organ systems has recently created interest in tissue engineering strategies. The structural and mechanical gradient present on tissues has its main focus at the interface since it seems that tissue-to-tissue interfaces are crucial for multitissue repair strategies. Thus, well-defined gradient model systems are of great importance in the study cell-matrix interactions at a biophysical level. It was hypothesized that by modulating the macromolecular enviroment, cells will assume a differential behaviour as a consequence of targeting specific intracellular signaling events leading to desirable cell fate patterning. Thus, cell viability and biofunctionality, as well differential paracrine secretory profiles and gene/cell marker expression, can be engineered by using human bone marrow stromal cells (hBMSCs).

METHODS: Hyaluronic acid and collagen type I formulations were produced at 1:1 ratio. Methacrylated Gellan-gum was produced and gradient hydrogels were assembled from bottom to top, layering each formulation % (w/v) sequentially. An intermediate cellular layer of hBMSCs was created. Rheology was used to assess the different mechanical properties. hBMSCs were observed under confocal microscopy for viability as well as analysis of cell morphology. ECM production as a function of cell number was evaluated. HIF-1α staining and O_2 concentration within the gradient evaluated the intrinsic hypoxic environment. Secretory cytokine measurement for angiogenesis and hypoxia factors was carried out using ELISA alongside gene and cell marker expression by RT-PCR and FACs analysis.

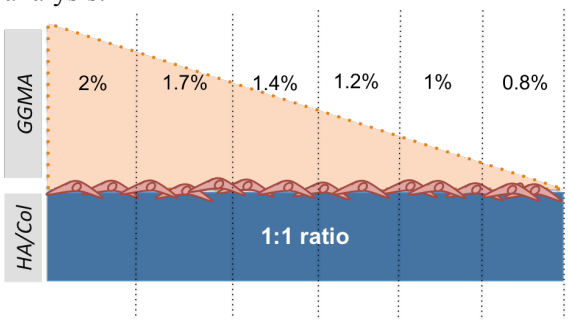

Fig. 1: Schematic representation of a tissue-to-tissue model system to differentially regulate human hBMSCs.

RESULTS: The gradient hydrogel was successfully constructed and validated by fluorescent emission of cells in the interface. Variations of oxygen concentrations were seen within a gradient over time and by HIF-1α staining. Significant differences in the levels of cytokine production in gradient hydrogels when compared to those of the control and over time were observed along with a differential genomic and cell marker expression of hBMSCs.

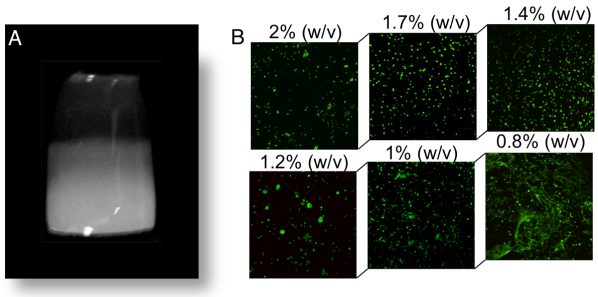

Fig. 2: (A) Image of a single hydrogel gradient; (B) hBMSCs viability was evaluated by the green emission of fluorescence for live staining (Calcein AM).

DISCUSSION & CONCLUSIONS: The macromolecular gradient hydrogel systems were successfully achieved demonstrating differentially cellular responses as a function of the macromolecules modulation.

ACKNOWLEDGEMENTS: This work has been supported by a research grant from Science Foundation Ireland (SFI), co-funded under the European Regional Development Fund under Grant Number 13/RC/2073 and also by the Portuguese Foundation for Science and Technology (SFRH/BD/81356/2011).